PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Factors associated with mortality among moderate and severe COVID 19 patients in India: A secondary analysis of a Randomised Controlled Trial
AUTHORS	Mammen, Joy; Kumar, Snehil; Thomas, Lovely; Kumar, Gunjan; Zachariah, Anand; Jeyaseelan, Lakshmanan; Peter, John Victor; Agarwal, Anup; Mukherjee, Aparna; Chatterjee, Pranab; Bhatnagar, Tarun; Rasalam, Jess; Chacko, Binila; Mani, Thenmozhi; Joy, Melvin; Rupali, Priscilla; Murugesan, Malathi; Daniel, Dolly; Latha, B; Bundas, Sunita; Kumar, Vivek; Dosi, Ravi; Khambholja, Janakkumar; de Souza, Rosemarie; Chander, B; Bahadur, Shalini; Dube, Simmi; Suri, Amit; Jindal, Aikaj; Shrivastav, Om; Barge, Vijay; Bajpayee, Archana; Malhotra, Pankaj; Singh, Neha; Tambe, Muralidhar; Sharma, Nimisha; Bhat, Shreepad; Kaulgud, Ram; Gurtoo, Anil; Reddy, Himanshu; Upadhyay, Kamlesh; Jain, Ashish; Patel, Tinkal; Nagori, Irfan; Jha, Pramod; Babu, KV; Aparna, C; Panjwani, Sunil; Natarajan, M; Baldi, Milind; Khadke, Vrushali; Dua, Seema; Singh, Ravindraa; Sharma, Ashish; Sharma, Jayashree; Gokhale, Yojana; Yadav, Pragya; Sapkal, Gajanan; Kaushal, Himanshu; Kumar, Saravana

VERSION 1 – REVIEW

REVIEWER	Schoenfeld, DA Massachusetts General Hospital, Biostatistics
REVIEW RETURNED	08-Mar-2021

GENERAL COMMENTS	It is quite difficult to use Cox model with time varying covariates in a situation where patients may no longer be available because they recover from the acute illness. The problem is that once patients recover and leave the hospital, their lab values are no longer available, so how to include them in the model is becomes difficult. These patients cannot be considered "censored" when they leave the hospital because they don't meet the fundamental requirement that censoring be independent of the outcome event, mortality. The paper did not describe what was done so I must presume that whatever was done was wrong, since this is a problem which requires careful attention. The Fine and Grey model might be more appropriate for this problem.

REVIEWER	Joyner, Michael Mayo Clinic, Department of Anesthesiology
REVIEW RETURNED	08-Apr-2021

GENERAL COMMENTS	This is a straight forward secondary analysis of the PLACID database that seeks to better understand how baseline risk factors and clinical status in COVID-19 patients interact and associated with mortality in an Indian population. The paper is clearly written and the outcomes contrasted to what is known in other countries and via other analyses. The take home message is that age, the presence of multiple co-morbidities, poor baseline clinical status, and high selected lab values interact and are associated with an increased risk of death. The one caveat I found most interesting is that early fever was associated with worse outcomes. There is very little to add to this paper and it will serve the medical community in India as they treat COVID patients and it will provide additional data to clinicians and scientists from across the world trying to understand outcomes in hospitalized COVID-19 patients.

REVIEWER	Anaya, Juan-Manuel Universidad del Rosario, Center for Autoimmune Diseases Research (CREA)
REVIEW RETURNED	21-Apr-2021

GENERAL COMMENTS	Authors describe the factors associated with mortality in patients with severe/critical COVID-19. Their results showed that comorbidities and levels of IL-6 were differential factors on admission between survivors and non-survivors. In addition, PaO2/FiO2 ratio, neutrophil lymphocyte ratio, D-Dimer levels, ferritin and LDH were predictors of mortality. Although this is of high interests and confirm previous work by others, some concerns exist aimed to improve the quality of this manuscript. Methods: 1. Definition of severity of COVID-19 should be revised. Authors argued that lower PaO2/FiO2 < 300 mmHg and SpO2 < 93% are criteria for “Moderate illness”. However, public health agencies, and clinical trails in COVID-19, consider this definition as “Severe disease”. 2. How the authors set the thresholds of laboratories used in regression analyses? As shown in the results, specific thresholds for each laboratory were used to estimate their relationship with outcomes. Please consider to be more specific on this issue. 3. As described in statistical analyses, authors included variables that were significantly different between groups in bivariate analyses, and additional others selected from literature review. However, it would be important to consider those variables with p values smaller than 0.25. This strategy may allow the identification of additional factors associated with mortality that are not easily identified in bivariate analyses. 4. Regarding the multiple testing for some laboratories. It would be of interest to consider generalized linear models (GLM) to analyze

	longitudinal data. That would show the behavior of those biomarkers over time based on the prespecified outcome (i.e., death – survival). Results: 1. Authors described in methods that they collected data in different points during the follow-up. A longitudinal analysis of those measurements (GLMs) is lacking 2. The description of thresholds for each laboratory should be provided. 3. Would it be possible to construct a clinical score based on results? That would help to translate the results to real-world clinical settings. This score could include the marginal probabilities for risk base on a score. This will be very helpful in shortage situations, in which ethical protocols must be established to select patients to be admitted in intensive care units. 4. Providing the bivariate analysis for treatments in both groups will increase the understanding of the manuscript Discussion: 1. After identification of factors associated with mortality what should a clinician do with the data? Although authors suggest that this study may help to ease the burden of the disease there is no mention about how it would happen. May the authors suggest some strategies based on the current evidence?

VERSION 1 – AUTHOR RESPONSE

Reviewer Comment	Authors reply
	Reviewer 1
It is quite difficult to use Cox model with time varying covariates in a situation where patients may no longer be available because they recover from the acute illness. The problem is that once patients recover and leave the hospital, their lab values are no longer available, so how to include them in the model is becomes difficult. These patients cannot be considered "censored" when they leave the hospital because they don't meet the fundamental	We thank you for clarifying the censoring concept and the use of Fine and Grey model in this situation. We have performed Fine and Gray regression model and incorporated the results in the manuscript.

requirement that censoring be independent of the outcome event, mortality. The paper did not describe what was done so I must presume that whatever was done was wrong, since this is a problem which requires careful attention. The Fine and Grey model might be more appropriate for this problem.	
	Reviewer 2
The one caveat I found most interesting is that early fever was associated with worse outcomes.	Thank you for your positive inputs on this manuscript
	Reviewer 3
Definition of severity of COVID-19 should be revised. Authors argued that lower PaO2/FiO2 < 300 mmHg and SpO2 < 93% are criteria for "Moderate illness". However, public health agencies, and clinical trials in COVID-19, consider this definition as "Severe diseases"	You are correct that although the initial plan was to include only patients with moderate illness, the current definitions encompass both patients with moderate and severe disease. Thus the title and the content have been modified to reflect this.
How the authors set the thresholds of laboratories used in regression analyses? As shown in the results, specific thresholds for each laboratory were used to estimate their relationship with outcomes. Please consider to be more specific on this issue.	We agree that one of the better ways of setting thresholds for laboratory parameters would be to do a Receiver Operating Characteristic (ROC) Curve and subsequently use the most appropriate "cut-off points". However in this study, we chose clinically relevant thresholds that would guide assessment of severity and therapy. This is clarified in Page 6 as, "For certain laboratory markers such as D-dimer, ferritin and LDH, clinically relevant thresholds were used for the analysis rather than using these data as continuous variables"

As described in statistical analyses, authors included variables that were significantly different between groups in bivariate analyses, and additional others selected from literature review. However, it would be important to consider those variables with p values smaller than 0.25. This strategy may allow the identification of additional factors associated with mortality that are not easily identified in bivariate analyses	We agree that we normally use thresholds of 0.20 or 0.25 in multivariate analysis. However, in view of multiple factors that were eligible for inclusion into the multivariable model, which would make the multivariable model challenging, the most significant and clinically relevant variables were used. Secondly, if we consider those variables with p values smaller than 0.25 the GLM was not converging. Therefore, we considered clinically and statistically important significant variables in the final model. We also wish to clarify that two alternative statistical methods are available to study survival analysis, namely the Cox model and the Fine and Gray model. One of the reviewers suggested the Fine and Gray model. After detailed review and biostatistics consultation, we felt that the Fine and Gary model was better suited for the analysis, with discharge as the competing event. We have therefore reanalysed our data using the Fine and Gray regression model and revised the manuscript accordingly.
Regarding the multiple testing for some laboratories. It would be of interest to consider generalized linear models (GLM) to analyse longitudinal data. That would show the behaviour of those biomarkers over time based on the pre specified outcome (i.e., death – survival).	We thank you for the suggestion. As advised we have done GLM analysis and the results are presented in table 1 at the end of the responses. Unfortunately in the GLM analysis, we were forced to keep the variables such as age, Neutrophil/Lymphocyte ratio, Platelet count, SOFA Score, D-dimer, Ferritin, CRP, LDH and PaO2/FiO2 as continuous variables, since GLM was not converging. The team felt that the GLM results are less informative as we would not be able to provide category specific risk for the above parameters. Moreover, we strongly believe that our data is kind of interval censoring. We have in addition, convergence problem with GLM-GEE analysis. Also we presume that due to short duration of follow up (28 days) period the GLM (GEE) and survival analysis will provide similar results. In view of the above, the utility of these results are limited in GEE as compared to survival analysis. Please refer table 1 below.
Authors described in methods that they collected data in different points during the follow-up. A longitudinal analysis of those measurements (GLMs) is lacking	We thank you for the suggestion. As advised we have done the GLM (GEE) analysis. In order to have the convergence we kept these variables as continuous (age, Neutrophil/Lymphocyte ratio, Platelet count, SOFA Score, D-dimer, Ferritin, CRP, LDH and PaO2/FiO2). Please refer the table 1 below.
The description of thresholds for each laboratory should be provided	This has been included in the text under statistical methods in Page 6 as, “The clinically relevant thresholds for these variables were set as

	>1.0 mg/L for D-dimer, ≥500 mg/mL for Ferritin and ≥450 IU/L for LDH.”
Would it be possible to construct a clinical score based on results? That would help to translate the results to real-world clinical settings. This score could include the marginal probabilities for risk base on a score. This will be very helpful in shortage situations, in which ethical protocols must be established to select patients to be admitted in intensive care units.	Yes, it is possible to construct a clinical score. However we felt that this would need much larger datasets. It would also require prospective validation subsequently.
Providing the bivariate analysis for treatments in both groups will increase the understanding of the manuscript	We are thankful for the input by reviewer. However, the primary study was done to see the effect of convalescent plasma in the mortality benefits of COVID -19 patient and was published. We did not find any such differences in mortality. This is a secondary analysis using the same data that was collected as part of the randomized trial. Other treatments regarding the usage of anticoagulants, immunomodulatory drugs and steroids were not guided by principal investigator and hence it varied across centres. Bivariate analysis for other drugs would be challenging given the multiplicity of treatments as well as the post hoc nature of such analysis, and may digress from the purpose of the study.
	Discussion
After identification of factors associated with mortality what should a clinician do with the data? Although authors suggest that this study may help to ease the burden of the disease there is no mention about how it would happen. May the authors suggest some strategies based on the current evidence?	We agree that there should be a clear understanding of what to do with the identification of factors associated with outcome. There are two key points that have come out of this study 1. Serial SOFA score may help with prognostication. The figure shows a clear divergence. Worsening SOFA score may identify the subset of patients who require more focused care 2. In terms of laboratory parameters, they would help in identifying subsets of patients who may benefit with anti-inflammatory or immunomodulatory treatment. This is summarized in the conclusion as, “A favourable outcome can be expected in moderately severe COVID. Older age, multiple comorbidities, low PaO2/FiO2 ratio and deranged inflammatory markers are associated with worse prognosis. Serial SOFA score can be used

	for prognostication. Understanding the symptoms, burden of comorbidities and systematic monitoring of key laboratory parameters offer opportunities for targeted intervention in COVID-19 with the use of anti- inflammatory or immunomodulatory agents.”

VERSION 2 – REVIEW

REVIEWER	Anaya, Juan-Manuel Universidad del Rosario, Center for Autoimmune Diseases Research (CREA)
REVIEW RETURNED	21-Jun-2021

GENERAL COMMENTS	Following are some comments aimed to improve the manuscript and focused on methods and results. Methods: 1. Regarding classification of participants, definition of “moderate illness” should be revised. As specified by the authors, all patients presented PaO2/FiO2 lower than 300. In this scenario, all patients should be considered as “Severe”. This classification should be reconsidered. 2. Was the adjustment by sex and age in the multivariate analysis based on differences between groups of mortality? If not, it would be unnecessary to include this type of adjustment in the model. Both variables are well-known risk factors for mortality. This could bias the results. Please, provide a short commentary in the Methods about this issue. 3. Decision of thresholds of D-Dimer and Ferritin was based on laboratory cut-off values? How these thresholds were defined? Please provide more information, including a reference. 4. Authors suggest that some variables were included in the multivariate analysis if they were strongly correlated with mortality in the “univariate analysis”, suggesting that this decision was not based on an exploratory bivariate analysis. How was the decision made? Please, clarify. 5. Please provide more information for the decision rules to include or exclude variables from the multivariate model. What do you mean by “strongly associated”? Methods require more detail to improve reproducibility and internal validity of results. 6. Did the authors explore different options to select critical variables associated with mortality on admission?

	7. Given the multiple testing implemented in this study. Bonferroni correction could have avoided the identification of false positive results on hypothesis testing. For example, it is likely that fever was not truly associated with mortality after correction (i.e., p value= 0.042). 8. Did the authors test for the role of variables on inclusion in the trial (day 0) in the prediction of mortality? Results: 1. Please avoid mentioning “univariate” instead of “bivariate” analysis. This may confuse the results. 2. Were the SOFA scores different on admission based on mortality? Was SOFA score on admission a predictor of mortality? 3. Please clarify the adjustment for competing risk factor, and its relevance for selecting those associated factors with mortality. 4. Did the treatments differ between groups? There is not information on the use of corticosteroids, antivirals or other medications that could bias the effect of risk factors on mortality.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3 Dr. Juan-Manuel Anaya, Universidad del Rosario	
METHODS	
1. Regarding classification of participants, definition of “moderate illness” should be revised. As specified by the authors, all patients presented PaO2/FiO2 lower than 300. In this scenario, all patients should be considered as “Severe”. This classification should be reconsidered	Thank you for the suggestions. We would like share the CLINICAL MANAGEMENT PROTOCOL FOR COVID-19 (for adults) by Government of India Ministry of Health and Family Welfare version 6, 24.05.21, which includes patients with SpO2 90 to ≤93% on room air, Respiratory Rate more or equal to 24 per minute under moderate illness and patient with respiratory rate >30 breaths/min, severe respiratory distress, SpO2 <90% on room air as severe diseases. https://www.mohfw.gov.in/pdf/ClinicalManagementProtocolforCOVID19.pdf The primary study was conducted based on criteria by the Government of India Ministry of Health and Family Welfare. In view of the above we have retained the classification as “moderate and severe”

2. Was the adjustment by sex and age in the multivariate analysis based on differences between groups of mortality? If not, it would be unnecessary to include this type of adjustment in the model. Both variables are well-known risk factors for mortality. This could bias the results. Please, provide a short commentary in the Methods about this issue.	Thank you for the suggestion and also we agree with your valuable comments. We have adjusted age and gender in the multivariable analysis. But it is not making any change in the results. So we didn't include gender in the final analysis. Please refer table 1 at the end of the response.
3. Decision of thresholds of D-Dimer and Ferritin was based on laboratory cut-off values? How these thresholds were defined? Please provide more information, including a reference.	The decision for the Ferritin and D dimer was based on laboratory cut-off values as well as literature review. The hemophagocytic lymphohistiocytosis (HLH)-2004 diagnostic criteria include Ferritin ≥500 µg/L. The clinical characteristics observed in the more severe cases of COVID-19 are reminiscent of HLH. Therefore, the cut-off was divided into two categories with a threshold of 500 µg/L. Similarly, pulmonary thromboembolism can be excluded in those with a D-dimer value < 500 µg/L. The thrombotic risk in the pulmonary vasculature is present before and during hospital admission in COVID-19 patients and hence the above cut-off was used. Several studies on COVID -19 and mortality assessment have shown ferritin levels >1500 ng/mL had higher odds of lethal outcome. https://doi.org/10.21203/rs.3.rs-143696/v1 10.1016/j.bjid.2021.101569 https://doi.org/10.3390/v13030419 https://www.nejm.org/doi/full/10.1056/NEJMoa1909159
4. Authors suggest that some variables were included in the multivariate analysis if they were strongly correlated with mortality in the "univariate analysis", suggesting that this decision was not	Two models were analyzed based on Laboratory parameters and Inflammatory Biomarkers values. The potential variables included in the models are based on statistical significance (Strictly by bivariate analysis of Fine and Gray's method) and clinical importance. However if a variable is expected to have collinear problem that was not included. For example Platelets. Also the variable which had sparse data, example vasopressor support and Invasive ventilation were not included due to convergence. The following has been included in the method section.

based on an exploratory bivariate analysis. How was the decision made? Please, clarify.	"The variables that are statistically significant or clinically important are considered in the multivariable analysis. However, if a variable is expected to have collinear concern or had sparse data that was not included in the analysis. There were two models built: (A) Laboratory Parameters and (B) Inflammatory Biomarkers".
5. Please provide more information for the decision rules to include or exclude variables from the multivariate model. What do you mean by "strongly associated"? Methods require more detail to improve reproducibility and internal validity of results.	Thank you for your suggestions, we have rewritten the method section as provided in response 4
6. Did the authors explore different options to select critical variables associated with mortality on admission?	No. Please refer response 4. We followed inclusion and exclusion criteria.
7. Given the multiple testing implemented in this study. Bonferroni correction could have avoided the identification of false positive results on hypothesis testing. For example, it is likely that fever was not truly associated with mortality after correction (i.e., p value= 0.042).	Inclusion of study variables in the model are based on Fine and Gray's method. Therefore fever was not included in the model. This is to kindly inform you that we have not used multiple testing methods such as Bonferroni correction in the analysis. Because this is applicable for a variable which has many categories. For example socio economic status that has got three categories. In such data Bonforroni can be done. When the variable fever has category Yes/No, Bonferroni cannot be done. Moreover each symptom was measured as Yes/No separately.
8. Did the authors test for the role of variables on inclusion in the trial (day 0) in	Yes, the role of variables on inclusion in the trial (day 0) was analysed but not necessarily be considered in the multivariable analysis.

the prediction of mortality?	
Reviewer: 3 Dr. Juan-Manuel Anaya, Universidad del Rosario	
RESULTS	
1. Please avoid mentioning “univariate” instead of “bivariate” analysis. This may confuse the results.	Thank you for suggestion. The terms univariate and bivariate has been omitted from the manuscript, to avoid confusion.
2. Were the SOFA scores different on admission based on mortality? Was SOFA score on admission a predictor of mortality?	The SOFA score reflects the clinical status at the time of enrolment into the PLACID TRIAL, based on inclusion criteria, which is different from the time of admission. Although we did not have SOFA score at admission, we did collect SOFA score over period of time, which seems to show a divergence between the two groups (Figure 1).
3. Please clarify the adjustment for competing risk factor, and its relevance for selecting those associated factors with mortality.	In time varying covariates in a situation where patients may no longer be available because they recover from the acute illness and leave the hospital, their laboratory values are no longer available. These patients cannot be considered "censored" when they leave the hospital because they don't meet the fundamental requirement that censoring be independent of the outcome event, mortality. Therefore, patients discharged and alive was considered as competing event and patients still admitted after day 28 (censored), whichever is earlier. Discharged alive was treated as a competing event because the event of discharged alive precludes the event of all-cause mortality. Reference: Competing risk A Practical Perspective by Melania Pintilie – Page no: 54
4. Did the treatments differ between groups? There is not information on the use of corticosteroids, antivirals or other medications that could bias the effect of risk factors on mortality.	Author is aware that there may be variability of treatment provided in the multiple centres, however, care was taken that patients received best standard of care for COVID-19 dictated by the best available evidence at the time and guidelines for the management of COVID-19 issued by health authorities of the Indian government. This has been included as one of the limitations in the study.

VERSION 3 – REVIEW

REVIEWER	Anaya, Juan-Manuel Universidad del Rosario, Center for Autoimmune Diseases Research (CREA)
REVIEW RETURNED	04-Aug-2021

GENERAL COMMENTS	All the queries were responded